baboon urate oxidase generated from the use of these primers has been named D3H baboon urate oxidase.

Total cellular RNA from pig and baboon livers was reverse-transcribed using a 1st strand kit (Pharmacia Biotech Inc. Piscataway, NJ). PCR amplification using Taq DNA polymerase (GibcoBRL, Life Technologies, Gaithersburg, MD) was performed in a thermal cycler (Ericomp, San Diego, CA) with the program [30 s, 95°C; 30 s, 55°; 60 s, 70°], 20 cycles, followed by [30 s, 95°C; 60 s, 70°] 10 cycles. The urate oxidase PCR products were digested with EcoRI and HindIII and cloned into pUC18 (pig), and were also cloned directly (pig and D3H baboon) using the TA cloning system (Invitrogen, Carlsbad, CA). cDNA clones were transformed into the *E. coli* strain XL1-Blue (Stratagene, La Jolla, CA). Plasmid DNA containing cloned uricase cDNAs was prepared and the cDNA insert sequence was analyzed by standard dideoxy technique. Clones that possessed the published urate oxidase DNA coding sequences (except for the D3H substitution in baboon urate oxidase described in Table I) were constructed and verified in a series of subsequent steps by standard recombinant DNA methodology.

The pig and D3H baboon cDNAs containing full length coding sequences were introduced into pET expression vectors (Novagen, Madison, WI) as follows. The D3H baboon uricase cDNA was excised from the TA plasmid with the NcoI and BamHI restriction enzymes and then subcloned into the NcoI and BamHI cloning sites of the expression plasmids pET3d and pET9d. Full length pig uricase cDNA was excised from a pUC plasmid clone with the EcoRI and HindIII restriction enzymes and subcloned into the EcoRI and HindIII sites of pET28b. The pig cDNA coding region was also introduced into the NcoI and BlpI sites of the expression plasmid pET9d after excision from the NcoI and BlpI sites of pET28b.

The pig-baboon chimera (PBC) cDNA was constructed by excising the 624 bp NcoI-ApaI restriciton fragment of D3H baboon uricase cDNA from a pET3d-D3H-baboon clone, and then replacing this D3H baboon segment with the corresponding 624 bp NcoI-ApaI restriciton fragment of pig cDNA. The resulting PBC urate oxidase cDNA consists of the pig urate oxidase codons 1-225 joined in-frame to codons 226-304 of baboon urate oxidase.

The pig-KS urate oxidase (PigKS) cDNA was constructed by excising the 864 bp NcoI-NdeI restriciton fragment of D3H baboon uricase cDNA from a pET3d-D3H baboon clone, and then replacing this D3H baboon segment with the corresponding 864 bp NcoI-NdeI restriciton fragment of pig cDNA. The resulting PKS urate oxidase cDNA consists of the pig urate oxidase codons 1-288 joined in-frame to codons 289-304 of baboon urate oxidase.

The amino acid sequences of the D3H baboon, pig, PBC, and PKS urate oxidases are shown in Figure 5 and the SEQUENCE LISTING). Standard techniques were used to prepare 15% glycerol stocks of each of these transformants, and these were stored at -70°C. When each of these species was expressed and the recombinant enzymes isolated (Table 2), the pig, PBC chimera, and PigKS uricases had very similar specific activity, which was approximately 4-5 fold higher than the specific activity of recombinant baboon uricase. This order was confirmed in several other experiments. The specific activity of PBC uricase prepared by several different procedures varied over a 2-2.5-fold range.

Table 2:

Comparison of Expressed Recombinant Mammalian Uricases

Construct	Specific Activity*	Relative Activity
	(Units/mg)	(Chimera=1)
PBC	7.02	1.00
PigKS	7.17	1.02
Pig	5.57	0.79
Baboon	1.36	0.19

* Protein was determined by the Lowry method. Uricase activity was determined spectrophotometrically (Priest and Pitts 1972). The assay was carried out at 23-25°C in a 1 cm quartz cuvette containing a 1 ml reaction mixture (0.1 M sodium borate, pH 8.6, 0.1 mM uric acid). Uric acid disappearance was monitored by decrease in absorbance at

292 nm. One international unit (IU) of uricase catalyzes the disappearance of one μmol of uric acid per minute.

E.coli BL21(DE3)pLysS transformants of the 4 uricase cDNA-pET constructs indicated in Table 2 were plated on LB agar containing selective antibiotics (carbenicillin and chloramphenicol for pET3d (pigKS); kanamycin and chloramphenicol for pET9d (PBC, pig, baboon)), as directed in the pET System Manual (Novagen, Madison WI). 5-ml cultures (LB plus antibiotics) were innoculated with single tranformant colonies and grown for 3 hours at 37°C. Then 0.1 ml aliquots were transferred to 100 ml of LB medium containing selective antibiotics and 0.1% lactose (to induce uricase expression). After overnight growth at 37°, bacterial cells from 0.5 ml aliquots of the cultures were extracted into SDS-PAGE loading buffer, and analyzed by SDS-mercaptoethanol PAGE: this established that comparable levels of uricase protein had been expressed in each of the 4 cultures (results not shown). The remaining cells from each 100 ml culture were harvested by centrifugation and washed in PBS. The cells were then re-suspended in 25 ml of phosphate-buffered saline, pH 7.4 (PBS) containing 1 mM AEBSF protease inhibitor (Calbiochem, San Diego, CA) and then lysed on ice in a Bacterial Cell Disruptor (Microfluidics, Boston MA). The insoluble material (including uricase) was pelleted by centrifugation (20,190 x g, 4°, 15 min). The pellets were washed twice with 10 ml of PBS, and then were extracted overnight at 4° with 2 ml of 1 M Na_2CO_3, pH 10.2. The extracts were diluted to 10 ml with water and then centrifuged (20,190 x g, 4°, 15 min). Uricase activity and protein concentrations were then determined.

EXAMPLE 2

Expression and isolation of recombinant PBC uricase (4 liter fermentor prep).

The pET3d-PBC uricase transformant was plated from a glycerol stock onto an LB agar plate containing carbenicillin and chloramphenicol, as directed in the Novagen pET System Manual. A 200 ml inoculum started from a single colony was prepared in